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Evaluation of Antioxidant Activity in Different Branded and Unbranded Honey

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Abstract: The metabolic extract of branded and unbranded honey samples were evaluated for their scavenging activity of DPPH free radical by using different concentrations (100, 200, 300, 500 and 600 µg/ml) of honey samples. The antioxidant activity of the extracts were determined against 1,1 -diphenyl-2-picryl hydroxyl (DPPH) by spectrophotometer. Sample 4 showed maximum antioxidant activity (84.33±1.23) at the concentration 600 µg/ml, whereas the lowest activity (24.12±1.17) was observed at the concentration 100 µg/ml in sample 3. In case of unbranded honey, sample 4 showed maximum antioxidant activity (81.26±1.44) at the concentration 600 µg/ml among all honey samples, whereas the lowest activity (20.22±1.19) was observed at the concentration 100 µg/ml in sample 1. As the concentration of these compounds increased the percent scavenging activity also increased. It is concluded from this study that all the branded and unbranded honey samples evaluated showed antioxidant activity. Branded samples presented better activity as compared to unbranded samples. Thus specifically honey could be used as alternative natural antioxidant in different formulations for food and pharmaceutical industries.

Key words: Honey · Branded · Unbranded · Antioxidant Activity

INTRODUCTION

Honey is natural, sweet and viscous fluid which produces by honey bees from the nectar of the flower. It has a complex mixture which composed mainly on carbohydrates, fructose, maltose, sucrose and glucose [1-2]. Honey also contain Vitamins such as thiamin (B1), riboflavin (B2), pyridoxine (B6), and ascorbic acid [3]. Honey has a long history of use as an effective medicine since ancient civilization for a wide range of disease conditions [4].

The physiological property of honey has been attributed to production of hydrogen peroxide formed by the enzyme glucose oxidase; antioxidant content, low pH value; osmotic action and a variety of enzymes [5]. One of the intrinsic features of honey is its antimicrobial property, which allows honey to be stored for a long period without becoming spoiled [6]. The anti-inflammation properties of honey have been known well [7].

Honey has been found to have the involvement of reactive oxygen species responsible for induction of inflammation [8]. When honey is applied to wounds, it effectively reduces the inflammation[9].A number of studies have firmly reinforced that honey is an effective medicinal treatment for burns and infected wounds[10].

Proper fueling of the liver is central to optimal glucose metabolism during sleep and exercise. Honey is Experimental evidence indicates that consumption of honey may improve blood sugar control and insulin sensitivity compared to other sweeteners. Honey has been shown to be a more effective cough suppressant for children than dextromethorphan Honey boosts immunity in healthy subjects, while sugar and artificial honey had either negative or very small beneficial effects, natural honey reduced total cholesterol 7%, triglycerides 2%, C-reactive protein 7%, homocysteine 6% and blood sugar 6% and increased HDL (good) cholesterol 2%[11-12]. Free radicals cause oxidative damage in many molecules, such as lipids, proteins and nucleic acids. Antioxidants possess free radical chain reaction breaking properties. which significantly delays or prevents oxidation of an oxidizable substrate when present in low concentration, including every type of molecules found *in vivo* [13].

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Honey also contain a number of pollen and wax which contain high amount of anti oxidant [14]. It mainly consist of phenolic acids, and polyphenols. phenolic acids are protocatequic acid, p- hydroxibenzonic acid, caffeic acid, chlorogenic acid, vanillic acid, p-coumaric acid, benzoic acid, ellagic acid, and cinnamic acid [15]. while the main components of flavonoids in honey are naringenin, kaempferol, apigenin, pinocembrin, chrysin, galangin, luteolin etc [16]. The flavonoids provid color, flavor to honey and have stronge anti-fungal, and antibacterial activity [17]. Antioxidant properties of honey act as an antidepressant during high emotional, physical and intellectual stress. Various antioxidant (polyphenols) are reported in honey have evolved as promising pharmacological agents in treatment of cancer [18] Keeping in view the importance of honey the present study was aimed to evaluate different branded and unbranded honey samples for its antioxidant activity collected from different areas of Khyber pakhtunkhwa Pakistan.

MATERIALS AND METHODS

Collection of Samples: Fifty branded and unbranded honey samples were purchased from local market from different locations of KPK Pakistan. After collections the samples were brought to Food Technology Centre, PCSIR Laboratories Complex Peshawar. All the samples in sealed containers were kept in refrigerator till analysis.

Chemical and Reagents: Methanol (Scharlau, Spain), DPPH (1,1-diphenyl-2-picryl hydroxyl) (Sigma Aldrich, Germany) and all the other chemicals and reagents used were of analytical grade

Antioxidant Activity: The scavenging of free radical of the extracts were determined against 1,1-diphenyl-2-picryl hydroxyl (DPPH) as reported [19-20].For stock solution 0.01g of each extract dissolved in 1ml of methanol and further diluted to five different concentrations (100-600 ìg/ml). Same dilutions were also made for ascorbic acid standard. One ml of each concentration was mixed thoroughly with freshly prepared DPPH solution and incubated for 10 minutes in dark at room temperature. After that, each sample was determined by the absorbance of UV at 517 nm wavelength for its antioxidant activity by the scavenging of free radical of DPPH. The Scavenging capacity of the sample was compared to that of control (1ml methanol + 2ml DPPH). The scavenging activity of the free radical of each sample expressed in percent inhibition using the given equation

$$\text{Percent (\%) inhibition of DPPH activity} = \{(Ab - As) / Ab)\} \times 100\ \%$$

Where Ab; represents the absorbance of the blank sample or control reaction and As; the absorbance of the test sample[21-22]. A curve of percent inhibition or percent scavenging effect against samples concentrations was plotted and the on the concentration where the scavenging reaches to 50% is its EC-50 value.

RESULTS

The methanolic extract of branded and unbranded honey samples were evaluated for their scavenging activity of DPPH free radical for different concentrations (100, 200, 300, 500 and 600 µg/ml) of honey samples. The activity in percent (%) of honey samples extracted in methanol and control (vitamin C standard) were presented (Table 1 and 2). These scavenging activities were proportional to the concentration of the extract. As the concentration of these compounds increased the percent scavenging activity also increased, when the scavenging reached to 50 % was its EC_{50} value.

This EC_{50} value inversely related to percent scavenging. The sample with lower EC_{50} value showed higher antioxidant activity [23]. On DPPH assay, the EC_{50} values of branded and unbranded samples were also

Table 1: DPPH radical scavenging activity of branded honey samples

Concentration (µg /ml)	Sample 1	Sample 2	Sample 3	Sample 4	Control (vitamin C)
100	24.45± 1.01	26.32±0.45	24.12±1.17	51.12±1.21	38.43±1.12
200	37.54±1.46	34.17±1.32	38.35±1.25	67.21±1.35	53.65±2.12
300	49.27±2.21	55.46±1.11	48.25±0.26	76.15±1.30	68.87±1.97
400	60.43±1.34	61.11±1.36	58.45±1.43	81.23±1.04	81.23±2.33
500	68.16±1.45	70.26±1.49	62.36±1.33	51.35±1.44	83.54±2.54
600	83.24±2.34	84.33±1.23	81.18±2.00	67.43±1.27	85.54±2.84

Mean ± S.D (n=3)

Table 2: DPPH radical scavenging activity of unbranded honey samples

Concentration (µg / ml)	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
100	20.22±1.19	25.43±1.11	24.32±1.13	26.27±1.29	38.43±1.12
200	28.34±1.14	38.16±1.23	34.14±1.26	41.23±1.36	53.65±2.12
300	35.12±1.16	51.28±1.25	41.33±1.35	54.31±1.13	68.87±1.97
400	43.35±1.23	65.35±1.07	55.12±1.24	64.04±1.22	81.23±2.33
500	51.25±1.34	73.13±1.27	62.19±1.00	76.13±1.43	83.54±2.54
600	57.01±1.22	80.42±1.32	81.24±1.37	81.26±1.44	85.54±2.84

Mean ± S.D (n=3)

Table 3: DPPH radical scavenging activity (EC_{50} in µg/g)

Sample	Sample 1	Sample 2	Sample 3	Sample 4	Control (vitamin C)
Branded	462	280	344	215	160
Unbranded	280	260	334	323	160

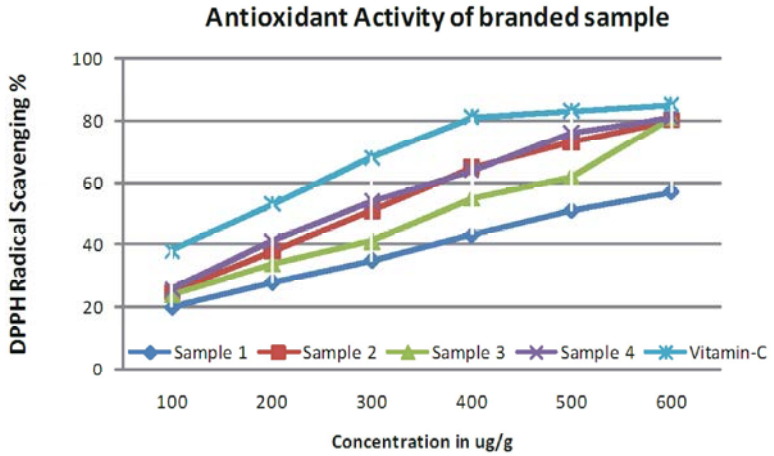

Fig. 1: Antioxidant Activity of Branded Honey Samples

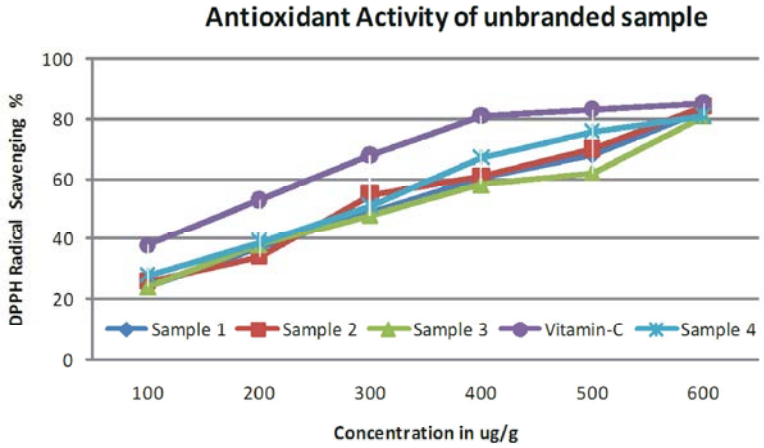

Fig. 2: Antioxidant activity of unbranded Honey samples

evaluated and presented (Table 3). It was observed that with increase in concentration of honey the free radical scavenging activity increase. The EC_{50} value was calculated from linear equation plotted from the different concentration of extracts against the percent scavenging (Fig 1 and 2).

DISCUSSION

DPPH is a free radical compound that has been currently used to determine the radical-scavenging ability of various compounds. It is a stable free radical which dissolves in methanol, has purple color and a

characteristic absorption at 517 nm. As antioxidants donate protons to this radical, the purple color from the DPPH assay solution becomes light yellow resulting in a decreases in absorbance. The decrease in absorbance is taken as a measure of the extent of radical scavenging [24-26].

The results showed that the branded sample of honey has an excellent DPPH radical scavenging activity as compared to unbranded samples. Sample 4 showed maximum antioxidant activity (84.33±1.23) at the concentration 600 µg/ml among four honey samples, whereas the lowest activity (24.12±1.17) was observed at the concentration 100 µg/ml in sample 3 (Table 1). In case of unbranded honey, sample 4 showed maximum antioxidant activity (81.26±1.44) at the concentration 600 µg/ml among all honey samples, whereas the lowest activity (20.22±1.19) was observed at the concentration 100 µg/ml in sample 1 (Table 2).

The data shows that by increasing the concentration of samples decreases the initial absorbance of DPPH. It was also noted that different phenolic contents including flavonols, flavones, isoflavonoids, phenolic acids and catechins were present in honey [13]. In case of branded honey samples the maximum EC_{50} values (462) was obtained for sample 1. Moderate EC_{50} values (280) and (344) were obtained for sample 2 and sample 3 respectively, while sample 4 showed the lowest value (215). In case of unbranded honey samples the maximum EC_{50} values (334) was obtained for sample 3. Moderate EC_{50} values (280) and (323) were obtained for sample 1 and sample 4 respectively, while sample 2 showed the lowest value (260). A lower value of EC_{50} indicates a higher antioxidant activity. EC_{50} values of branded honey samples were lower then unbranded honey samples.

CONCLUSION

It is concluded from this study that all the branded and unbranded honey samples evaluated showed antioxidant activity. Branded samples presented better activity as compared to unbranded samples. Thus specifically honey may constitute a suitable source and could be used as alternative natural antioxidant in different formulations for the preparation of food and pharmaceutical products, which is very well evidenced by the present work.

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